

Research Paper

Allelic variation of low molecular weight glutenin subunits composition and the revealed genetic diversity in durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf))

Xin Hu^{1,4}, Yanchun Peng¹, Xifeng Ren¹, Junhua Peng², Eviatar Nevo³, Wujun Ma⁴ and Dongfa Sun^{*1,5}

¹) College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, China

²) Science and Technology Center, China National Seed Group Co., Ltd., Wuhan, 430075, Hubei, China

³) Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31905, Israel

⁴) Australia-China Joint Centre for Wheat Improvement, State Agriculture Biotechnology Centre, School of Veterinary and Life Sciences, Murdoch University, WA 6150, Australia

⁵) Hubei Collaborative Innovation Center for Grain Industry, Jingzhou, 434025, Hubei, China

Low molecular weight glutenin subunits (LMW-GS) play an important role in determining the bread-making characteristics of dough in the end-use quality of wheat. In this study, A total of 149 worldwide-originated durum wheat were used to analyze the composition of LMW-GS using MALDI-TOF-MS. Based on the allelic variation of glutenin subunits, the genetic diversity was evaluated for the 149 durum wheat. Five types of alleles were identified at the *Glu-A3* locus with *Glu-A3e*, *Glu-A3a/c*, *Glu-A3f*, *Glu-A3d* and *Glu-A3b* accounting for 43.0%, 16.1%, 12.8%, 10.1% and 7.4 % of the accessions, respectively. Five types of alleles were identified at the *Glu-B3* locus: *Glu-B3d* (60.4%), *Glu-B3b* (6.0%), *Glu-B3c* (6.0%), *Glu-B3h* (2.7%) and *Glu-B3f* (0.7%). Two novel alleles encoding abnormal subunits 40500 Da and 41260 Da were identified at the *Glu-A3* and *Glu-B3* loci, respectively. Further studies are needed to match these novel alleles to previously discovered novel alleles. Moreover, the genetic diversity analysis indicated that great genetic variation existed in durum wheat among encoding loci of glutenin subunits, released periods of varieties and different geographical origins. The results provide more important information of potential germplasm for the improvement of durum wheat and common wheat.

Key Words: durum wheat, MALDI-TOF-MS, low molecular weight glutenin subunits (LMW-GS), allelic variation, genetic diversity.

Introduction

Glutenin proteins, the compositions of wheat flour, play a key role in determining wheat rheological characteristics including dough strength and extensibility and bread-making performance (Bekes *et al.* 2001, Butow *et al.* 2003, Ma *et al.* 2005). Glutenin fractions consist of aggregated proteins linked by interchain disulfide bonds, and the polymeric glutenin proteins have various sizes ranging in molecular weight from less than 300,000 Da to more than 1,000,000 Da (Liu *et al.* 2010, Wieser *et al.* 2006, Wieser 2007). Glutenin subunits could be divided into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (D'Ovidio and Masci 2004,

Jackson *et al.* 1983, Payne and Corfield 1979). It has been recognized that the molecular weight (MW) distribution of glutenins mainly determines the properties and baking performance of dough (Weegels *et al.* 1996).

LMW-GS contain a large amount of polypeptides. For the difficult to distinguish LMW-GS from gliadins, the composition, structure of LMW-GS and the relationship between LMW-GS and grain processing quality have not yet been studied to the same level as the HMW-GS (Appelbee *et al.* 2009, D'Ovidio and Masci 2004). LMW-GS, significant components of wheat storage proteins, are important in determining dough properties (including gluten strength and dough extensibility) (Cornish *et al.* 2001, Gianibelli *et al.* 2001). Therefore, identifying the allelic variation of LMW-GS and analyzing the relationships between LMW-GS and grain processing quality have been an attractive research area on quality improvement for the last 20 years, and the successful utilization of specific LMW-GS alleles is foundational and essential for quality breeding programs (Békés

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*Corresponding author (e-mail: sundongfa1@mail.hzau.edu.cn)

et al. 2006, Gupta *et al.* 1994, He *et al.* 2005).

LMW-GS were initially identified from the extracts of wheat flour by gel filtration and starch gel electrophoresis (Elton and Ewart 1966). Most LMW-GS are encoded by the *Glu-A3*, *Glu-B3* and *Glu-D3* loci on the short arms of chromosomes 1A, 1B and 1D, respectively (where, *Glu-A3* and *Glu-B3* in tetraploid wheat), and tightly linked to the complex *Gli-1* loci, which encode γ - and ω -gliadins (Anderson *et al.* 2009, Payne *et al.* 1984, Pogna *et al.* 1990, Singh and Shepherd 1988). A few LMW-GS were encoded by the *Glu-A3* locus on chromosome 1A, however, there is wide variation for LMW-GS encoded by *Glu-B3* locus on chromosome 1B in common wheat (Gupta and Shepherd 1990, Liu *et al.* 2010, Yan *et al.* 2003). Although the *Glu-D3* locus has less variation with five alleles initially reported by Gupta and Shepherd (1990), discrepancy exists among different studies about the alleles (Appelbee *et al.* 2009, Ikeda *et al.* 2006, Jackson *et al.* 1996), suggesting that further studies are necessary to clarify the genetic variation at this locus.

One-dimensional SDS-PAGE, 2-DE (two-dimensional gel electrophoresis (IEF \times SDS-PAGE)) and HPLC (High performance liquid chromatography) methods have been generally used to identify and select specific HMW-GS and LMW-GS with superior quality in many breeding programs (Dworschak *et al.* 1998, Yahata *et al.* 2005). Matrix-assisted laser desorption/ionization time-of flight (MALDI-TOF-MS) is an effective and very important approach in rapidly and easily identifying glutenin subunits for its high accuracy and sensitivity in analyzing samples, which has been particularly useful in wheat quality breeding programs (Dworschak *et al.* 1998, Elfatih *et al.* 2013, Liu *et al.* 2009, 2010, Peng *et al.* 2016, Zheng *et al.* 2011). MALDI-TOF-MS has widely been used to identify the HMW-GS compositions of common landraces of bread wheat collected from the Yangtze-River region of China (Zheng *et al.* 2011), to detect the compositions of HMW-GS in durum wheat from different countries (Elfatih *et al.* 2013), to establish an analytical standard for identifying LMW-GS using a set of 19 near-isogenic lines (NIL) of cultivar Aroona (Wang *et al.* 2015).

Durum wheat (*Triticum durum* Desf.) is a tetraploid species containing A and B genomes ($2n = 4x = 28$, AABB) (Peng *et al.* 2011), and is the main material of semolina for the processing of pasta, bagel and other local end-products of Mediterranean (Fabriani *et al.* 1988, Nachit *et al.* 1992). The quality of durum wheat end-products depends mainly on glutenin composition. Different composition of HMW-GS and LMW-GS and their combinations may result in differences in gluten elasticity and strength (Elfatih *et al.* 2013). Generally, the LMW-GS are associated with resistance and extensibility of dough (Cornish *et al.* 2001, Metakovsky *et al.* 1990), and some allelic forms of LMW-GS present even greater effects than HMW-GS on these characteristics (Gupta *et al.* 1994, Payne *et al.* 1987). LMW-GS are also important for the end-use quality of dough in durum wheat, especially subunits encoded by loci on chromo-

some 1B (D'Ovidio and Masci 2004, Josephides *et al.* 1987). *LMW-2*, a specific allele encoding typical LMW-GS, is associated with the best pasta making characteristics (Payne *et al.* 1984), and also seems to be significant in determining bread-making properties (D'Ovidio and Masci 2004, Peña *et al.* 1994). Generally, as the genetic basis of modern wheat cultivars is narrow, special durum wheat cultivars, containing unusually useful genes are rich resources for wheat quality improvement (Li *et al.* 2006). The aims of the present study were to: (a) identify the LMW-GS compositions of worldwide-originated durum wheat using MALDI-TOF-MS, and reveal the difference of the LMW-GS compositions in different accessions, and (b) evaluate the genetic diversity in world-wide origin durum wheat based on the allelic variation of LMW-GS and HMW-GS, and genetic diversity in different released periods of varieties and geographical origins, respectively.

Materials and Methods

Plant materials

A total of 149 accessions of worldwide-originated durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.), $2n = 4x = 28$, AABB) were used in this study, including 25 from East Asia (EA), 24 from West Asia (WA), 33 from Europe (EU), 16 from Africa (AF), 32 from North America (NA), 12 from South America (SA), and 7 from Australia (AU) (Table 1). The accessions used in the present study were also included in the study of Elfatih *et al.* (2013), and were all obtained from USDA (United States Department of Agriculture).

Protein extraction

Proteins were extracted from 20 mg whole meal based on the sequential procedure of Singh *et al.* (1991). The samples were extracted with 1.0 ml of 55% propanol-1-ol (v/v) for 5 min vortexing, followed by incubation for 20 min at 65°C, then continued vortexing for 5 min with a centrifugation at $10,000 \times g$ for 5 min. Repeated this step three times to completely remove the gliadins. The glutenin in the pellet was reduced with 55% propanol-1-ol, containing 0.08 M Tris-HCl solution and 1% dithiothreitol (DTT) and incubation for 30 min at 65°C, followed by addition of 1.4% v/v of 4-vinylpyridine, and alkylation and incubation overnight at room temperature. For MALDI-TOF-MS analysis, 80% acetone was used to precipitate the LMW-GS portion.

MALDI-TOF-MS

The dried compounds of LMW-GS samples were dissolved in 60 μ l acetonitrile (ACN)/H₂O (v/v, 50:50) containing 0.05% v/v trifluoroacetic acid (TFA) for 1 h at room temperature. Referring to the dried droplet method of Kussmann *et al.* (1997), sample preparation was carried out using sinapinic acid (SA) as matrix. The matrix solution was made by dissolving SA in ACN/H₂O (50:50 v/v) containing 0.05% TFA (v/v) at a concentration of 10 mg/ml. Mixing the extracted LMW-GS solution (a total of 60 μ l)

Table 1. The LMW-GS compositions for 149 accessions analyzed by MALDI-TOF-MS

| Code | Accession identifier ^a | Accession name | Regions | Place of origin | Year of collection | Type | <i>Glu-A3</i> | <i>Glu-B3</i> |
|------|-----------------------------------|-------------------|---------|---------------------------------|--------------------|----------|---------------|---------------|
| H45 | PI 233213 | Sevindz | EA | Azerbaijan | 1956 | Cultivar | 40503 Da | d |
| H61 | PI 345707 | Sevindz | EA | Azerbaijan | 1969 | Cultivar | 40494 Da | d |
| H1 | Cltr 11495 | Wash. No. 2628 | EA | Heilongjiang, China | 1932 | Cultivar | b | d |
| H14 | Cltr 5077 | FHB4495 | EA | China | 1916 | Landrace | e | b |
| H142 | PI 70658 | Tulatai Maitai | EA | Heilongjiang, China | 1926 | Landrace | d | h |
| H143 | PI 70662 | Lumanian | EA | Heilongjiang, China | 1926 | Landrace | d | 41300 Da |
| H146 | PI 74830 | ICARDA-IG-82496 | EA | Jiangsu, China | 1927 | Landrace | a/c | d |
| H147 | PI 79900 | N-85 | EA | Heilongjiang, China | 1929 | Landrace | d | 41325 Da |
| H15 | Cltr 5083 | FHB4501 | EA | China | 1916 | Landrace | a/c | f |
| H16 | Cltr 5094 | FHB4512 | EA | Beijing, China | 1916 | Landrace | d | 41259 Da |
| H19 | Cltr 8327 | Suifu | EA | Sichuan, China | 1924 | Landrace | e | d |
| H23 | PI 124292 | ICARDA-IG-82575 | EA | Jiangsu, China | 1937 | Landrace | f | d |
| H54 | PI 283853 | China 34 | EA | China | 1962 | Cultivar | e | d |
| H90 | PI 435100 | Bian Sui | EA | China | 1979 | Cultivar | e | d |
| H92 | PI 447421 | ST-33 | EA | Xinjiang, China | 1980 | Cultivar | f | d |
| H84 | PI 41015 | Jalalia | EA | Madhya Pradesh, India | 1915 | Landrace | b | d |
| H85 | PI 41342 | Hansia Broach | EA | Gujarat, India | 1915 | Landrace | b | d |
| H133 | PI 61351 | Medea | EA | Hokkaido, Japan | 1924 | Landrace | d | 41291 Da |
| H134 | PI 61352 | Roumania | EA | Hokkaido, Japan | 1924 | Landrace | d | 41289 Da |
| H130 | PI 61112 | Cltr 7395 | EA | Kazakhstan | 1924 | Landrace | a/c | 41248 Da |
| H131 | PI 61123 | Cltr 7406 | EA | Kazakhstan | 1924 | Landrace | 40511 Da | 41284 Da |
| H32 | PI 176228 | ICARDA-IG-84631 | EA | Nepal | 1949 | Landrace | b | d |
| H41 | PI 210910 | T 1 | EA | Punjab, Pakistan | 1953 | Cultivar | a/c | d |
| H42 | PI 210911 | T 2 | EA | Punjab, Pakistan | 1953 | Cultivar | a/c | d |
| H83 | PI 388132 | FAO 33.268 | EA | Punjab, Pakistan | 1974 | Landrace | a/c | d |
| H123 | PI 591959 | DW 1 | WA | Cyprus | 1994 | Cultivar | e | d |
| H43 | PI 210952 | Damluko | WA | Cyprus | 1953 | Landrace | f | d |
| H47 | PI 237632 | Tripolitico | WA | Cyprus | | Cultivar | e | d |
| H25 | PI 140184 | ICARDA-IG-82637 | WA | Khuzestan, Iran | 1941 | Landrace | e | c |
| H44 | PI 222675 | ICARDA-IG-85523 | WA | East Azerbaijan, Iran | 1954 | Landrace | a/c | d |
| H48 | PI 243790 | ICARDA-IG-85615 | WA | Tehran, Iran | 1957 | Landrace | e | d |
| H56 | PI 289821 | ICARDA-IG-97583 | WA | Fars, Iran | 1963 | Landrace | e | c |
| H144 | PI 70736 | ICARDA-IG-82459 | WA | Iraq | 1926 | Landrace | e | b |
| H28 | PI 165846 | Amarah | WA | Iraq | 1948 | Cultivar | f | b |
| H37 | PI 208903 | Rash Kool | WA | Iraq | 1953 | Landrace | e | d |
| H38 | PI 208907 | Lara | WA | Iraq | 1953 | Landrace | e | d |
| H39 | PI 208908 | Mendola | WA | Iraq | 1953 | Landrace | a/c | d |
| H40 | PI 208910 | Sin El-Jamil | WA | Iraq | 1953 | Landrace | e | 41259 Da |
| H51 | PI 253801 | K918 | WA | Ninawa, Iraq | 1958 | Landrace | e | d |
| H49 | PI 249816 | N-163 | WA | Israel | 1958 | Cultivar | e | d |
| H50 | PI 249820 | Neveh Yaar 51 | WA | Israel | 1958 | Cultivar | e | 41269 Da |
| H57 | PI 292035 | | WA | Israel | 1963 | Cultivar | e | c |
| H81 | PI 384043 | Merarit | WA | Israel | 1973 | Cultivar | 40643 Da | c |
| H82 | PI 388035 | Line 76 | WA | Israel | 1974 | Cultivar | e | b |
| H105 | PI 520415 | Syrian Durum 27 | WA | Syria | 1987 | Cultivar | e | d |
| H24 | PI 134596 | Fere-Alexandrinum | WA | Syria | 1939 | Landrace | e | d |
| H33 | PI 182697 | Nashabie | WA | Dimashq, Syria | 1949 | Landrace | a/c | d |
| H36 | PI 193391 | Aleppo | WA | Halab, Syria | 1951 | Landrace | b | 41267 Da |
| H26 | PI 152567 | Aden | WA | Yemen | 1945 | Cultivar | a/c | h |
| H109 | PI 546462 | Gergana | EU | Khaskovo, Bulgaria | 1990 | Cultivar | 40580 Da | d |
| H60 | PI 344743 | Apulicum 233 | EU | Bulgaria | 1969 | Cultivar | e | 41254 Da |
| H72 | PI 352450 | | EU | France | 1969 | Cultivar | d | 41283 Da |
| H12 | Cltr 2468 | | EU | Germany | 1904 | Landrace | 40472 Da | d |
| H58 | PI 306664 | Heines Hartveizen | EU | Lower Saxony, Germany | 1965 | Cultivar | f | d |
| H64 | PI 352389 | Caravicos | EU | Greece | 1969 | Cultivar | f | d |
| H124 | PI 593005 | V. 433 | EU | Latium, Italy | 1996 | Cultivar | f | d |
| H68 | PI 352408 | T-1560 | EU | Italy | 1969 | Cultivar | e | d |
| H69 | PI 352415 | Aziziah 17/45 | EU | Latium, Italy | 1969 | Cultivar | b | d |
| H115 | PI 56233 | Cltr 7041 | EU | Lisboa, Portugal | 1923 | Cultivar | f | d |
| H74 | PI 376498 | DF 14/71 | EU | Romania | 1972 | Cultivar | a/c | d |
| H75 | PI 376500 | DF 31/71 | EU | Romania | 1972 | Cultivar | a/c | d |
| H76 | PI 376501 | DF 42/71 | EU | Romania | 1972 | Cultivar | a/c | 41292 Da |
| H77 | PI 376509 | DF 4/72 | EU | Romania | 1972 | Cultivar | 40617 Da | d |
| H78 | PI 376511 | DF 6/72 | EU | Romania | 1972 | Cultivar | a/c | b |
| H79 | PI 376512 | DF 7/72 | EU | Romania | 1972 | Cultivar | a/c | d |
| H13 | Cltr 3267 | Chistunka | EU | Altay, Russian Federation | 1911 | Landrace | d | 41227 Da |
| H132 | PI 61189 | Cltr 7472 | EU | Krasnoyarsk, Russian Federation | 1924 | Landrace | e | d |
| H70 | PI 352436 | T-2114 | EU | Former Soviet Union | 1969 | Cultivar | d | h |
| H71 | PI 352437 | T-2115 | EU | Former Soviet Union | 1969 | Cultivar | 40503 Da | b |

Table 1. (continued)

| Code | Accession identifier ^a | Accession name | Regions | Place of origin | Year of collection | Type | <i>Glu-A3</i> | <i>Glu-B3</i> |
|------|-----------------------------------|--------------------------------|---------|-----------------------------|--------------------|----------|---------------|---------------|
| H67 | PI 352404 | Torcal | EU | Spain | 1969 | Cultivar | 40499 Da | d |
| H35 | PI 192711 | Ostpreuss | EU | Gotland, Sweden | 1950 | Cultivar | e | d |
| H63 | PI 352377 | T-357 | EU | Switzerland | 1969 | Cultivar | a/c | d |
| H111 | PI 560702 | TU85-008-10-2 | EU | Siirt, Turkey | 1986 | Landrace | e | d |
| H112 | PI 560717 | TU85-054-01-2 | EU | Bitlis, Turkey | 1986 | Landrace | e | 41267 Da |
| H113 | PI 560718 | TU85-054-02 | EU | Bitlis, Turkey | 1986 | Landrace | e | d |
| H114 | PI 560889 | TU86-24-02-2 | EU | Siirt, Turkey | 1989 | Landrace | f | c |
| H21 | PI 109588 | T-538 | EU | Ankara, Turkey | 1935 | Cultivar | 40491 Da | 41252 Da |
| H62 | PI 346985 | Hacimestan | EU | Turkey | 1970 | Cultivar | e | d |
| H52 | PI 278223 | Gartons Early Cone | EU | England, United Kingdom | 1962 | Cultivar | e | c |
| H53 | PI 278648 | ICARDA-IG-85863 | EU | England, United Kingdom | 1962 | Cultivar | e | b |
| H59 | PI 321702 | Nursi | EU | England, United Kingdom | 1967 | Cultivar | e | d |
| H91 | PI 438973 | Har'kovskaja 51 | EU | Kharkiv, Ukraine | 1980 | Cultivar | d | 41274 Da |
| H107 | PI 546060 | DT367 | NA | Saskatchewan, Canada | 1990 | Cultivar | e | d |
| H108 | PI 546362 | DT369 | NA | Saskatchewan, Canada | 1991 | Cultivar | e | d |
| H11 | Cltr 17337 | Wakooma | NA | Saskatchewan, Canada | 1974 | Cultivar | e | d |
| H119 | PI 583724 | 8682-D051-NG | NA | Saskatchewan, Canada | 1994 | Cultivar | e | d |
| H120 | PI 583731 | G8973-AG1-G | NA | Saskatchewan, Canada | 1994 | Cultivar | e | d |
| H121 | PI 583732 | G8973-AG1-NG | NA | Saskatchewan, Canada | 1994 | Cultivar | e | d |
| H122 | PI 583733 | G8973-AQ1-G | NA | Saskatchewan, Canada | 1994 | Cultivar | e | d |
| H98 | PI 519751 | D 31729-2L-OL | NA | Federal District, Mexico | 1987 | Cultivar | e | 41274 Da |
| H101 | PI 519761 | Maghrebi'S' | NA | Federal District, Mexico | 1987 | Cultivar | e | 41298 Da |
| H102 | PI 519866 | CB 088 | NA | Federal District, Mexico | 1987 | Cultivar | f | d |
| H103 | PI 520053 | 31814-1L-OC | NA | Federal District, Mexico | 1987 | Cultivar | e | 41287 Da |
| H104 | PI 520173 | Tal | NA | Mexico | 1987 | Cultivar | e | 41291 Da |
| H129 | PI 610765 | CIGM91.347-6 | NA | Federal District, Mexico | 1999 | Cultivar | f | d |
| H135 | PI 634315 | Canelo | NA | Federal District, Mexico | 2001 | Cultivar | e | d |
| H136 | PI 634318 | Afuwan | NA | Federal District, Mexico | 2001 | Cultivar | e | d |
| H30 | PI 168708 | Barrigon Glabrous Selection | NA | Mexico | 1948 | Cultivar | b | h |
| H6 | Cltr 15874 | D 19329-28M-11Y | NA | Mexico | 1972 | Cultivar | a/c | d |
| H86 | PI 422289 | Maghrebi 72 | NA | Mexico | 1978 | Cultivar | e | 41304 Da |
| H88 | PI 428453 | Dommel'S' | NA | Federal District, Mexico | 1978 | Cultivar | f | d |
| H99 | PI 519752 | D 31648-2L-OL | NA | Federal District, Mexico | 1987 | Cultivar | d | 41304 Da |
| H110 | PI 560335 | KS91WGRC14 | NA | Kansas, United States | 1992 | Cultivar | e | d |
| H118 | PI 573005 | Imperial | NA | Arizona, United States | 1988 | Cultivar | f | d |
| H125 | PI 600931 | D-5003 | NA | California, United States | 1982 | Cultivar | e | d |
| H126 | PI 601250 | Westbred Laker | NA | Arizona, United States | 1985 | Cultivar | e | d |
| H137 | PI 656793 | NSGC 19376 | NA | California, United States | 2009 | Cultivar | e | 41307 Da |
| H138 | PI 656794 | IR51-8 | NA | California, United States | 2009 | Cultivar | e | 41325 Da |
| H139 | PI 656795 | IR17-47 | NA | California, United States | 2009 | Cultivar | e | 41317 Da |
| H150 | PI 9872 | Galgalos | NA | Erevan, Armenia | 1903 | Cultivar | f | b |
| H18 | Cltr 6881 | Akrona | NA | Colorado, United States | 1923 | Cultivar | d | 41268 Da |
| H2 | Cltr 12068 | Kubanka 314 | NA | North Dakota, United States | 1940 | Cultivar | 40490 Da | 41264 Da |
| H3 | Cltr 13246 | Ramsey | NA | North Dakota, United States | 1955 | Cultivar | d | 41255 Da |
| H4 | Cltr 13333 | Wells | NA | North Dakota, United States | 1957 | Cultivar | e | 41253 Da |
| H116 | PI 565259 | Yurac Mexico | SA | Cochabamba, Bolivia | 1991 | Landrace | e | d |
| H117 | PI 565266 | Mexico | SA | Cochabamba, Bolivia | 1991 | Landrace | e | d |
| H100 | PI 519759 | D 73121 | SA | Brazil | 1987 | Cultivar | e | 41214 Da |
| H34 | PI 191645 | Timor | SA | Sao Paulo, Brazil | 1950 | Cultivar | e | d |
| H10 | Cltr 17159 | CAR 1234 | SA | La Araucania, Chile | 1972 | Cultivar | a/c | d |
| H7 | Cltr 17057 | CAR 1131 | SA | La Araucania, Chile | 1972 | Cultivar | a/c | d |
| H8 | Cltr 17058 | CAR 1132 | SA | La Araucania, Chile | 1972 | Cultivar | a/c | d |
| H9 | Cltr 17157 | CAR 1232 | SA | La Araucania, Chile | 1972 | Cultivar | a/c | d |
| H55 | PI 286546 | Morocho Colorado | SA | Pichincha, Ecuador | 1963 | Cultivar | e | d |
| H148 | PI 91956 | Chumpe Negro | SA | Junin, Peru | 1931 | Cultivar | a/c | d |
| H149 | PI 92024 | Candeal | SA | Cajamarca, Peru | 1931 | Cultivar | d | d |
| H29 | PI 168692 | Muestra 2 Barba Blanca Anquipa | SA | Peru | 1948 | Cultivar | f | d |
| H22 | PI 11715 | Marouani | AF | Mascara, Algeria | 1904 | Landrace | a/c | d |
| H106 | PI 532119 | 2515 | AF | Minufiya, Egypt | 1988 | Cultivar | f | d |
| H127 | PI 60712 | Gawi | AF | Egypt | 1924 | Landrace | f | c |
| H128 | PI 60742 | Sinai No. 8 | AF | Sinai, Egypt | 1924 | Landrace | b | d |
| H141 | PI 7016 | Mishriki | AF | Alexandria, Egypt | 1901 | Landrace | b | c |
| H145 | PI 7422 | Girgeh | AF | Sawhaj, Egypt | 1901 | Landrace | b | c |
| H27 | PI 153774 | Durum H | AF | Giza, Egypt | 1946 | Cultivar | f | d |
| H66 | PI 352395 | T-1303 | AF | Ethiopia | 1969 | Cultivar | f | b |
| H73 | PI 352551 | Abyssinicum | AF | Ethiopia | 1969 | Landrace | d | 41252 Da |
| H87 | PI 42425 | Zwartbaard | AF | South Africa | 1916 | Landrace | 40508 Da | d |
| H93 | PI 45442 | ICARDA-IG-98118 | AF | Free State, South Africa | 1917 | Landrace | 40546 Da | d |
| H94 | PI 45443 | ICARDA-IG-98119 | AF | Cape Province, South Africa | 1917 | Landrace | 40552 Da | d |

Table 1. (continued)

| Code | Accession identifier ^a | Accession name | Regions | Place of origin | Year of collection | Type | <i>Glu-A3</i> | <i>Glu-B3</i> |
|------|-----------------------------------|----------------|---------|------------------------------|--------------------|----------|---------------|---------------|
| H95 | PI 46766 | Golden Ball | AF | Cape Province, South Africa | 1918 | Cultivar | e | 41308 Da |
| H65 | PI 352390 | T-842 | AF | Tunisia | 1969 | Cultivar | e | d |
| H96 | PI 51210 | Mahmoudi | AF | Tunisia | 1920 | Landrace | e | d |
| H97 | PI 519380 | BD 1645 | AF | Tunisia | 1987 | Cultivar | e | 41258 Da |
| H140 | PI 67341 | Huguenot | AU | Western Australia, Australia | 1926 | Cultivar | 40514 Da | d |
| H17 | Cltr 5136 | Indian Runner | AU | Victoria, Australia | 1916 | Landrace | 40497 Da | d |
| H20 | PI 107606 | Cadia | AU | Australia | 1934 | Cultivar | b | 41259 Da |
| H31 | PI 174645 | Huguenot | AU | Western Australia, Australia | 1949 | Cultivar | a/c | d |
| H46 | PI 235159 | Giza | AU | New South Wales, Australia | 1956 | Cultivar | e | 41260 Da |
| H80 | PI 377882 | Duramba | AU | Australia | 1973 | Cultivar | e | d |
| H89 | PI 428701 | AUS 20299 | AU | Australia | 1978 | Cultivar | e | d |

^a The accession identifier is adopted from the USDA.ARS National Plant Germplasm System-Germplasm Resources Information Network (https://www.ars-grin.gov/npgs/acc/acc_queries.html).

with SA solution (1:10 v/v) for protein-SA mixture, and 2 µl of this mixture was deposited on to a 96-sample MALDI target probe tip, then dried at room temperature. MALDI-TOF-MS experiments were performed on a Voyager DE-PRO TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA) with UV nitrogen laser (337 nm) at the State Agriculture Biotechnology Center, Murdoch University, Australia. Analyses were performed with the following parameters: acceleration voltage 25 kV and delay time 900 ns, mass range 10,000–50,000 Da. The low mass gate value (10,000 m/z) for analysis was chosen to avoid saturation of the sensor. The new standard established with 16 single *Glu-3* allele substitution lines of Aroona, 25 gene deletion lines and 60 wheat lines with known LMW-GS compositions as reference in Wang *et al.* (2015), was used to analyze the composition of LMW-GS alleles. The established standard in Wang *et al.* (2015) for specific MALDI-TOF spectrum patterns corresponding to LMW-GS allele were summarized in Supplemental Table 1.

Genetic diversity analysis

The genetic diversity was evaluated based on the allelic variation of LMW-GS in this study and HMW-GS in the study of Elfatih *et al.* (2013) (see Supplemental Table 2). POWERMARKER Ver. 3.25 (Liu and Muse 2015) was used to analyze the genetic diversity using the genetic parameters Nei's gene diversity and polymorphism information content (PIC). A phylogenetic NJ tree based on accessions and regions were performed by POWERMARKER Ver. 3.25 with 1000 bootstrap replicates. A consensus tree with bootstrap values was reconstructed by the consensus program of PHYLIP (Plotree and Plotgram 1989) and displayed by FigTree Ver.1.4 (Rambaut 2014).

Results

Allelic variation of LMW-GS at *Glu-A3* and *Glu-B3*

According to the established standard in Wang *et al.* (2015) for specific MALDI-TOF spectrum patterns corresponding to LMW-GS alleles (Supplemental Table 1), the

mass spectra of the LMW glutenin subunits showed well-separated peaks in the spectrum of each material, and the mass spectra of the LMW glutenin subunits for some materials were shown in Fig. 1. The LMW-GS compositions for 149 accessions analyzed by MALDI-TOF-MS are listed in Table 1. A total of 12 alleles (ten previously reported and two unreported alleles) of LMW-GS were found in the MALDI-TOF-MS profile and their frequencies were presented in Table 2. A total of 23 types of LMW-GS compositions were detected during 149 accessions at *Glu-A3* and *Glu-B3* loci (Table 3).

At the *Glu-A3* locus, five previously reported alleles were identified. *Glu-A3e* showed the highest frequency that was detected in 43.0% of the 149 accessions, followed by the *Glu-A3a/c* (16.1%), *Glu-A3f* (12.8%), *Glu-A3d* (10.1%) and *Glu-A3b* (7.4%) (Tables 1, 2). However, alleles *Glu-A3a* and *Glu-A3c* have identical molecular masses, and were difficult to be distinguished by MALDI-TOF-MS (Wang *et al.* 2015). Moreover, one previously unreported allele was detected at *Glu-A3* locus in sixteen (10.7%) accessions encoding a novel subunit with a molecular weight of approximately 40,500 Da (ranging from 40,472 Da to 40,580 Da).

At the *Glu-B3* locus, five previously reported alleles were identified. Out of 149 accessions, 60.4% (90) of them were identified with *Glu-B3d*, indicating that *Glu-B3d* was the most frequent allele at *Glu-B3* locus. *Glu-B3b* and *Glu-B3c* each accounted for 6.0% of the accessions. *Glu-B3h* was detected in 4 accessions and *Glu-B3f* was detected only in one accession. Moreover, a new LMW glutenin subunit was identified with the molecular weight of around 41,260 Da (ranging from 41,214 Da to 41,325 Da) in 36 accessions (24.2% of the accessions examined) (Tables 1, 2).

A total of 23 types of LMW-GS compositions were detected in this study. The most common combination type is *Glu-A3e* + *Glu-B3d* (26.2%), followed by *Glu-A3a/c* + *Glu-B3d* (12.8%), *Glu-A3e* + a new subunit with molecular weight of about 41260 Da (11.2%), moreover the combination of a new subunit with a molecular weight of about 40,500 Da and *Glu-B3d* was detected in 11 accessions

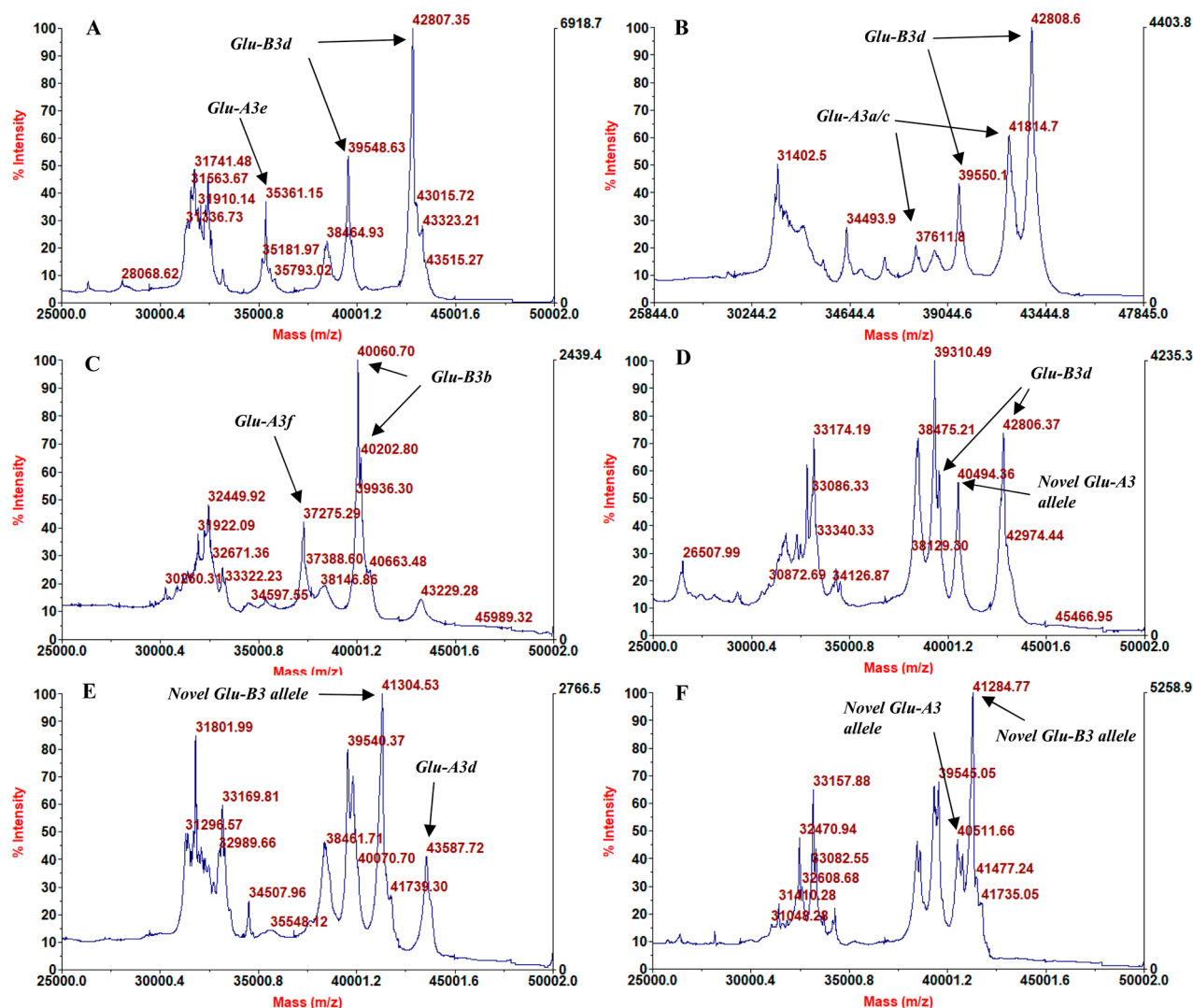


Fig. 1. Detection of LMW-GS for some durum accessions by MALDI-TOF-MS. Accessions code: (A) H24, (B) H39, (C) H66, (D) H61. (E) H99, (F) H131.

Table 2. Allele frequencies of LMW-GS revealed by MALDI-TOF-MS

| Locus | LMW-GS | Number | Frequency % |
|--------------|----------|--------|-------------|
| <i>GluA3</i> | 40500 Da | 16 | 10.7 |
| | a/c | 24 | 16.1 |
| | b | 11 | 7.4 |
| | d | 15 | 10.1 |
| | e | 64 | 43.0 |
| | f | 19 | 12.8 |
| <i>GluB3</i> | 41260 Da | 36 | 24.2 |
| | b | 9 | 6.0 |
| | c | 9 | 6.0 |
| | d | 90 | 60.4 |
| | f | 1 | 0.7 |
| | h | 4 | 2.7 |

(Table 3). Different subunits and different combinations of subunits have different effects on the quality and processing quality of the dough.

Overall, 12 alleles (ten previously reported and two unreported alleles) of LMW-GS were found in the MALDI TOF-MS at the two loci in durum wheat. Two unreported alleles were observed at loci *Glu-A3* and *Glu-B3*, with 10.7% for *Glu-A3* and 24.2% for *Glu-B3*. Furthermore, we also detected, in some materials, the spectrum peaks of approximately 43,267 Da and 41,758 Da, which were reported to be associated with novel subunits in Wang *et al.* (2015). However, these peaks were not novel in the current study.

Genetic diversity

The genetic diversity is listed in Table 4. For LMW-GS coding loci, a higher genetic diversity was detected at *Glu-A3* locus with Nei's gene diversity, and PIC values of 0.245 and 0.208, respectively, while 0.225 and 0.186 for *Glu-B3* locus, respectively. For HMW-GS coding loci, the genetic diversity of *Glu-A1* (with Nei's gene diversity, and

Table 3. Allele combinations and variants at *Glu-A3* and *Glu-B3* loci in durum wheat

| | <i>GluA3</i> | <i>GluB3</i> | Number | Frequency % |
|----|--------------|--------------|--------|-------------|
| 1 | 40500 Da | 41260 Da | 3 | 2.0 |
| 2 | 40500 Da | b | 1 | 0.7 |
| 3 | 40500 Da | c | 1 | 0.7 |
| 4 | 40500 Da | d | 11 | 7.4 |
| 5 | a/c | 41260 Da | 2 | 1.3 |
| 6 | a/c | b | 1 | 0.7 |
| 7 | a/c | d | 19 | 12.8 |
| 8 | a/c | f | 1 | 0.7 |
| 9 | a/c | h | 1 | 0.7 |
| 10 | b | 41260 Da | 2 | 1.3 |
| 11 | b | c | 2 | 1.3 |
| 12 | b | d | 6 | 4.0 |
| 13 | b | h | 1 | 0.7 |
| 14 | d | 41260 Da | 12 | 8.1 |
| 15 | d | d | 1 | 0.7 |
| 16 | d | h | 2 | 1.3 |
| 17 | e | 41260 Da | 17 | 11.4 |
| 18 | e | b | 4 | 2.7 |
| 19 | e | c | 4 | 2.7 |
| 20 | e | d | 39 | 26.2 |
| 21 | f | b | 3 | 2.0 |
| 22 | f | c | 2 | 1.3 |
| 23 | f | d | 14 | 9.4 |

Table 4. The genetic diversity of *GluA3*, *GluB3*, *GluA1* and *GluB1* based on LMW-GS and HMW-GS alleles

| Locus | Genetic Diversity | PIC |
|--------------|-------------------|-------|
| <i>GluA3</i> | 0.245 | 0.208 |
| <i>GluB3</i> | 0.225 | 0.186 |
| <i>GluA1</i> | 0.309 | 0.249 |
| <i>GluB1</i> | 0.153 | 0.134 |

PIC values of 0.309 and 0.249, respectively) was higher than *Glu-B1* (with Nei's gene diversity, and PIC values of 0.153 and 0.134, respectively).

The genetic diversity for the 7 geographical regions is shown in **Table 5**. European accessions showed the highest values of both Nei's gene diversity (0.216) and PIC (0.181), followed by African (AF: 0.213, 0.175), East Asian (EA: 0.206, 0.172) and North American accessions (NA: 0.195, 0.159), while the lowest level of Nei's gene diversity and PIC were detected in South American accessions (SA: 0.156, 0.128). West Asian (WA) and Australian (AU) accessions had a moderate level of Nei's gene diversity and PIC (with the values of 0.191, 0.160 and 0.180, 0.145, respectively).

The difference of genetic diversity between landrace and cultivar, and the release time is shown in **Table 6**. The higher genetic diversity was detected in the cultivars with Nei's gene diversity and PIC values of 0.215 and 0.180, than values in the landrace. Therefore, according to Ren *et al.* (2013), the cultivars were also further divided into three temporal groups: OC (old cultivars before 1965), EGR (early green revolution, 1966–1980), PGR (post green revolution, 1980–2009), to compare the genetic difference. The genetic diversity parameters of three temporal groups of cultivars are shown in **Table 6**. Loss of genetic diversity

Table 5. The genetic diversity of the accessions from 7 ecogeographic regions based on LMW-GS and HMW-GS alleles

| Origin | Genetic Diversity | PIC |
|--------|-------------------|-------|
| AF | 0.213 | 0.175 |
| AU | 0.180 | 0.145 |
| EA | 0.206 | 0.172 |
| EU | 0.216 | 0.181 |
| NA | 0.195 | 0.159 |
| SA | 0.156 | 0.128 |
| WA | 0.191 | 0.160 |

Table 6. Comparison of genetic diversity generated by the allelic variation of LMW-GS and HMW-GS between landraces and cultivars

| Group | Genetic Diversity | PIC |
|------------------------|-------------------|-------|
| Cultivar | 0.215 | 0.180 |
| Landrace | 0.210 | 0.175 |
| Time group of Cultivar | | |
| Before 1965 | 0.239 | 0.200 |
| 1965–1980 | 0.211 | 0.177 |
| 1981–2009 | 0.165 | 0.135 |

was observed from OC to EGR (Nei's gene diversity: 0.239 vs. 0.211 and PIC values: 0.200 vs. 0.177). However, the decrease of genetic diversity was observed from EGR to PGR (Nei's gene diversity: 0.200 vs. 0.177 and PIC values: 0.165 vs. 0.135).

Cluster analysis

The allelic variation of LMW-GS and HMW-GS loci was used for the cluster analysis. The consensus NJ tree of accessions based on Nei's genetic distance (Nei 1972) is shown in **Fig. 2**. The durum wheat accessions were divided into two major groups.

Group I contained the American accessions (North America and South America), this group was dominated by landraces and cultivars released during OC, EGR and PGR. Group II was further divided into 7 subgroups, grouping of some accessions appeared to be associated with the release period of varieties to some extent (**Fig. 2**, **Supplemental Table 3**).

The consensus NJ tree was constructed based on geographical regions of accessions (**Fig. 3**). The result indicated that the accessions of AU was different from the other regions. The accessions from other regions were divided into two group, EA, EU and AF were clustered in one group, WA, SA and NA were in the other group.

Discussion

Allelic variation of LMW-GS at *Glu-A3* and *Glu-B3* and the novel subunits

The allelic variation of glutenin subunits can provide a more direct, reliable and efficient tool for the conservation and management of germplasm. In this study, the compositions and allelic variation of low molecular weight glutenin subunit (LMW-GS) in 149 worldwide-originated durum wheat were analyzed using MALDI-TOF-MS.

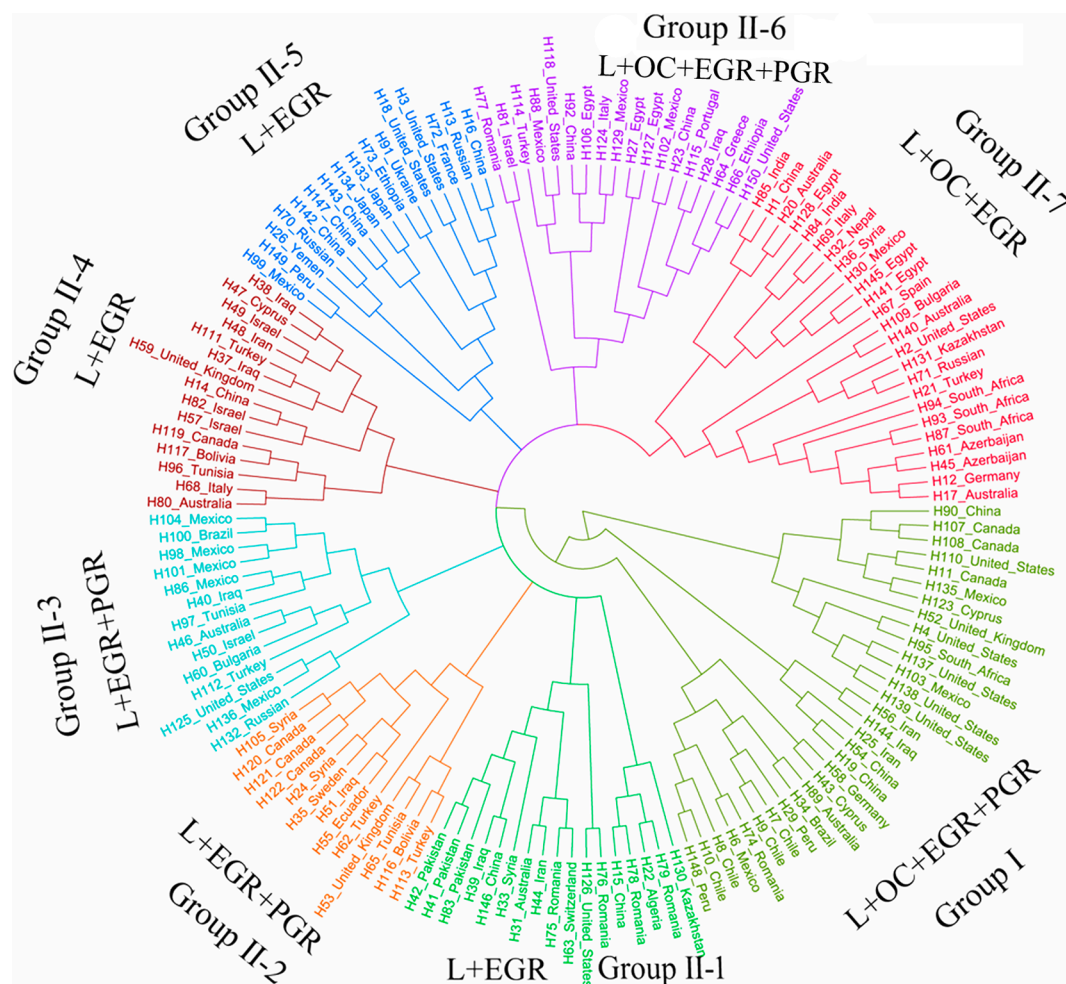


Fig. 2. The NJ tree of 149 durum accessions based on the Nei's genetic distance calculated from the alleles of LMW-GS and HMW-GS. The allelic variation data of HMW-GS was from the study of Elfatih *et al.* (2013), L: Landrace, OC: Old cultivars before 1965, EGR: Early green revolution, 1966–1980, PGR: Post green revolution, 1980–2009.

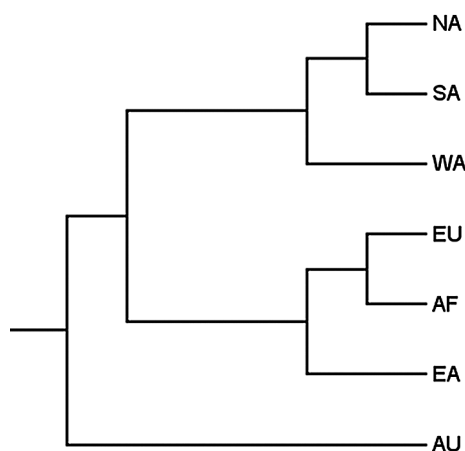


Fig. 3. The consensus NJ tree for the accessions from 7 ecogeographic regions based on the Nei's genetic distance calculated from the alleles of LMW-GS and HMW-GS. The allelic variation data of HMW-GS was from the study of Elfatih *et al.* (2013).

For the *Glu-A3* locus, the most frequent allele was *Glu-A3e* accounting for 43.0%, while the frequency of *Glu-A3a/c* alleles was lower (16.1%). This is different from some previous studies. Bellil *et al.* (2012), Bradová and Štočková (2010), and Nieto-Taladriz *et al.* (1997) reported that *Glu-A3a/c* was the predominant alleles in wheat, while *Glu-A3e* was relatively low. *Glu-A3a* and *Glu-A3c* appeared to be world widely predominant among bread wheat in previous studies, whereas, *Glu-A3e* was predominant among durum wheat in our collections. However, low frequency of *Glu-A3c* was found in the Algerian local and old durum wheat cultivars (Cherdouh *et al.* 2005, Hamdi *et al.* 2010). Different species (common wheat and durum wheat), different sources and distributions of materials should lead to the differences in allele frequencies of LMW-GS reported by different scientists. It seems that the frequency of *Glu-A3a* and *Glu-A3c* were higher in common wheat than in durum wheat, while the frequency of *Glu-A3e* was relatively low. A previous study discovered that *Glu-A3e* reduced the maximum resistance and extensibility of dough in relative to

other alleles of *Glu-A3* (Appelbee 2007). It is worthy of noting that the *Glu-A3d* is a desirable allele for gluten quality and pan bread quality (He *et al.* 2005) and presented in 15 landraces. Moreover, a novel allele, encoding a subunit with a molecular weight of approximately 40,500 Da (ranging from 40,472 Da to 40,580 Da) located at *Glu-A3*, was detected in 20 accessions.

Allelic variation at the *Glu-A3* locus did not significantly affect gluten strength, whereas the *Glu-B3* locus had a significant influence on gluten strength, as measured by sedimentation volume on durum wheat (Vazquez *et al.* 1996). For the *Glu-B3* locus, five previously reported alleles were identified in our study. The most frequent allele was *Glu-B3d* (60.4%). The similar result was reported in Saharan bread wheat and Durum wheat from Algerian Oases by Bellil *et al.* (2012). However, *Glu-B3d* had medium to weak dough properties, and should be avoided at the early stages of a bread wheat breeding program (Luo *et al.* 2001). *Glu-B3b* was rare and only detected in 9 accessions accounting for 6%, which is consistent with the studies of Bellil *et al.* (2010, 2012). It is worthy of noting that a novel allele, expressing a subunit with a molecular weight of approximately 41,260 Da (ranging from 41,214 Da to 41,325 Da) at *Glu-B3*, presented in 60 accessions.

Following the standard for LMW-GS of common wheat varieties reported by Wang *et al.* (2015), we were able to identify the alleles of LMW-GS in most of the durum wheat accessions. Most LMW-GS compositions of durum wheat materials can be detected rapidly and easily according to the characteristic peaks of standard samples in Wang *et al.* (2015). Several novel alleles were identified in landraces collected from Yangtze-River region of China in our research and in Peng *et al.* (2016) and Wang *et al.* (2015) at *Glu-A3* and *Glu-B3* loci. It should be mentioned that Peng *et al.* (2016) and Wang *et al.* (2015) found two novel subunits associated with the spectrum peaks 41,758 Da at *Glu-A3* and 40,499 Da at *Glu-B3*. In our research, we also detected the spectrum peaks with similar masses of approximately 41,758 Da and 40,499 Da. However, compared with the results of Wang *et al.* (2015), our data tended to indicate the spectrum peak of approximately 41,758 Da present with the characteristic spectrum peak (37,600 Da) of *Glu-A3a/c*. This might suggest that the spectrum peak 41,758 Da was another characteristic spectrum peak for *Glu-A3a/c* (Fig. 1B). The spectrum peak 40,499 Da was identified as a characteristic spectrum peak for subunit of a novel allele at *Glu-B3* in Peng *et al.* (2016) and Wang *et al.* (2015), however, this characteristic peak can be confidently treated as a new allele located at *Glu-A3* in our study (Fig. 1D, 1F). Furthermore, another novel allele encoding a subunit with a molecular weight of approximately 41,260 Da at *Glu-B3* locus was detected in our study, which was not reported in their studies (Fig. 1E, 1F). A more detailed study is needed to identify the novel alleles in the landraces collected from the Yangtze-River region in China and worldwide-originated durum wheat. Recently, a set of PCR primers have been de-

veloped and effectively used to amplify the coding region of the HMW-GS and LMW-GS genes, and numerous LMW-GS genes have been identified in the *Glu-A3*, *Glu-B3* and *Glu-D3* coding regions (Lan *et al.* 2013, Si *et al.* 2014, Wang *et al.* 2012). Using the conserved primers, the novel LMW-GS gene sequences may be amplified from genomic DNA of wheat accessions to match the novel alleles to previously reported alleles.

Genetic diversity

The genetic diversity of *Glu-A3* was higher than *Glu-B3* in this set of durum wheat, similar results were reported in the study of Moragues *et al.* (2006) for the accessions from North Africa, South Europe and West Asia. However, the genetic diversity of *Glu-A1* was higher than *Glu-B1* in this study, which was opposite to the result of Moragues *et al.* (2006). This could be due to different materials. In the study of Moragues *et al.* (2006), only 63 durum wheat landraces from the Iberian Peninsula and other Mediterranean countries were analyzed, while in our study, more world-wide originated accessions (including landraces and cultivars released in different period) were used.

The genetic diversity of durum wheat from 7 ecogeographic regions revealed by the allelic variation of LMW-GS and HMW-GS indicated the genetic diversity of durum wheat from ecogeographic origins was different. Generally, great genetic variation should exist in the center of origin and domestication. It was reported that “Fertile Crescent” is the centers of origin and diversification of durum wheat (Vavilov 1951). However, in this study, the highest genetic diversity of durum wheat was found in EU accessions, followed by AF and EA accessions, while WA accessions showed moderate levels of genetic diversity. Similar result was reported by Ren *et al.* (2013) based on SNP markers. One of the reasons should be uneven distribution of landraces or cultivars among countries and different genetic diversity levels between landraces and cultivars used in this study as discussed by Ren *et al.* (2013). Moreover, the genetic diversity, revealed by the allele variation of LMW-GS and HMW-GS loci, should be different to the genetic diversity evaluated by SNP markers around genome, this should be another reason.

The difference of genetic diversity between landrace and cultivar had been reported by Ren *et al.* (2013) based on SNP markers. In our study, the difference of genetic diversity based on the allele variation of LMW-GS and HMW-GS loci showed similar results to Ren *et al.* (2013) on some extent. The higher genetic diversity was detected in cultivar than landrace. Decrease of genetic diversity was observed from OC (before 1965) to EGR (1965–1980), which was consisted to Ren *et al.* (2013). As discussed in Ren *et al.* (2013), the low level diversity of varieties released in 1965–1980 (EGR) might be due to the “Early Green Revolution”, which resulted from widely use of the semi-dwarf varieties and the high yield breeding target. While, a continuous loss of genetic diversity was observed from EGR (1965–1980)

to PGR (1981–2009), which is opposite to the result of Ren *et al.* (2013). During PGR, CIMMYT have realized the danger of narrowing down genetic diversity, they changed the breeding strategy for increasing genetic diversity of wheat and durum wheat, which increased genetic diversity (Reeves 1999). However, meanwhile, CIMMYT started to focus on the quality breeding, although, the genetic diversity was generally increased considering the whole genome. While the quality related loci or regions of chromosome were suffered selection pressure in breeding programs, and single germplasms with high-quality subunits was selected by breeder for breeding and promoting, these result in the decreasing of genetic diversity observed from EGR (1965–1980) to PGR (1981–2009) on the allele variation of LMW-GS and HMW-GS loci in this study.

Cluster analysis

Cluster analyses for accessions and their geographical originations were performed based on the allelic variation of LMW-GS and HMW-GS loci (Figs. 2, 3). some of accessions from the same geographic region and release period were clustered together though into different groups corresponding to their geographical regions of collection, release period and accession type (Landrace or Cultivar) (Fig. 2, Supplemental Table 3). For example, Group I contained 11 accessions (Cultivar) from NA (North America), most of which (8/11) were released during PGR (Fig. 2, Supplemental Table 3), and Group II-7 contained 6 landraces, all of which were from AF (Africa) and collection before 1924 (Fig. 2, Supplemental Table 3). These results indicated that many of the accessions were clustered corresponding to their geographical regions, collection time and accession type, which may be due to the similar environmental conditions or the utilization of single elite germplasm in breeding or agronomical practices.

The NJ tree for the origination regions of the durum accessions showed that the accessions from EA, EU and AF were close to each other, and accessions from WA, SA and NA have close relationship (Fig. 3). The accessions of AU were much difference from the others. A close relationship of between EA, EU and AF accessions align well the discussion of Moragues *et al.* (2006) based on the accepted theory of wheat cultivation spreading across the Mediterranean basin (Feldman and Millet 2001, Zohary *et al.* 2012), the theory reported *T. monococcum* spread west from the Fertile Crescent by two ways: North, through the Balcan Peninsula, Greece and Italy, and south through ancient Egypt. This explained the close relationship among the accessions of EA, EU and AF. The close relationship among the accessions of WA, SA and NA indicated that the three geographic regions maybe share some similar origin germplasms with similar allelic variation of LMW-GS and HMW-GS. Moreover, the germplasm exchange through cultural diffusion or historical human dispersal could also play an important role. As we known, between the Old and New World after Columbus' voyages, not only the European culture, but also many crops

(including durum wheat landraces and cultivars) were introduced from Europe to the America (Capparelli *et al.* 2005). Besides, trade routes and immigration between WA, SA and NA, new varieties of wheat were transported or shared. This maybe also explain the closer relationship among the accessions of WA, SA and NA on some aspect.

In conclusion, the results of allelic variation of LMW-GS provide useful information for wheat breeder to explore germplasm resources for end-use quality improvement. Further studies of the two novel alleles are currently underway to match them with previously reported alleles and to evaluate their potential utility value in improving the bread-making quality. The genetic diversity indicated that despite strict selection pressures on cultivar purity and related breeding practices, there is still a significant level of genetic variation on LMW-GS and HMW-GS alleles in the modern varieties of durum wheat. And there existed abundant genetic variation among loci, released periods of varieties and different geographical origins. The results provide useful information of potential germplasm for the improvement of durum wheat and common wheat.

Acknowledgments

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